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(54) Title: USE OF TYROSINE KINASE INHIBITORS FOR TREATING INFLAMMATORY DISEASES

(57) Abstract: The present invention relates to a method for treating inflammatory diseases such as rheumatoid arthritis (RA), comprising administering a tyrosine kinase inhibitor to a human in need of such treatment, more particularly a non-toxic, selective and potent c-kit inhibitor. Preferably, said inhibitor is unable to promote death of IL-3 dependent cells cultured in presence of IL-3.

Use of tyrosine kinase inhibitors for treating inflammatory diseases

The present invention relates to a method for treating inflammatory diseases such as
5 rheumatoid arthritis (RA), comprising administering a tyrosine kinase inhibitor to a human in need of such treatment, more particularly a non-toxic, selective and potent c-kit inhibitor. Preferably, said inhibitor is unable to promote death of IL-3 dependent cells cultured in presence of IL-3.

10 Millions of people are afflicted with rheumatoid arthritis in the USA alone, which represent approximately 1% of the total adult population (Mitchell, D. 1985. Rheumatoid Arthritis, eds. J.B. Lippincott Co., Philadelphia, pp. 133-150). This progressive inflammatory disease is very important both socially and economically because it leads to the disabling and extensive suffering of individuals. The distribution
15 of affected and deformed joints typically involves the small articulations of the hands and feet, although the larger appendicular joints like the shoulders and hips are often affected in established disease.

Current methods for treating rheumatoid arthritis include administration of non-steroidal
20 anti-inflammatory drugs such as acetylsalicylic acid (aspirin), ibuprofen, naproxen, cox2 inhibitors and other agents such as penicillamine, methotrexate, cytotoxic agents (e.g., azothrioprine), 4-aminoquinoline agents, and immunomodulators. However, no satisfactory treatment is available as of today. In addition, many of the therapeutic agents administered to alleviate pain and inflammation associated with the disease, such as
25 disease-modifying antirheumatic drugs (DMARDs) and non-steroidal anti-inflammatory agents (NSAIDs), produce intolerable side effects. The use of nonspecific immunosuppressive drugs which suppress the entire immune system has also been

proposed but it increases the risk of infection. Furthermore, prolonged use of such drugs can entail severe toxic side effects and is only partially effective in mitigating symptoms of rheumatoid arthritis.

- 5 Therefore, improved treatments of rheumatoid arthritis, which can suppress symptoms such as inflammation, swelling, abnormal neovascularization, bone erosion, or cartilage erosion are needed.

10 The cause of rheumatoid arthritis remains elusive but it is now generally accepted that infectious agents or toxins, genetic susceptibility, and immune or autoimmune responses play direct or indirect roles. After initiation of the disease process, it is believed that activated T cells and their products are responsible for the progressive destruction of articular cartilage and sub-chondral bone that is characteristic of rheumatoid arthritis.

15 The symptoms of the disease result from a massive increase in the number of cells lining the synovium of the joint. The various cell types which are present include type A synoviocytes, which have the characteristics of monocytes or terminally differentiated macrophages, and type B synoviocytes which are fibroblast-like. As these cells increase in number, the continuous inflammation causes initial symptoms. Eventually, local release of enzymes by the synovial internal lining degrade the extracellular matrix and 20 cause deformity. Panayi et al, 1992, Arth. Rheum., 35, pp.729-735 mentioned that rheumatoid factors is caused by a cell-mediated process involving T cells, antigen-presenting cells (APC), macrophages, synoviocytes, and cytokines. T cells (Panayi et al., 1992, Arthritis Rheum. 35, 729-735), B cells (Zvaifler, 1973, Adv. Immunol. 265, 265-336), and other leukocytes such as dendritic cells, macrophages and neutrophils (Thomas 25 and Lipsky, 1996, Arthritis Rheum. 39, 183-190) have been shown to play a role the pathogenesis of this inflammatory disease. Rheumatoid arthritis is also generally considered an autoimmune disease that is thought to be associated with activity of

autoreactive T cells (Harris E., 1990, The New England Journal of Medicine, 322: 1277-1289).

Cytokines have also been indicated as being implicated in the pathophysiology of rheumatoid arthritis. Tumor necrosis factor (TNF-a) has received attention because it is consistently found in synovium of patients suffering from rheumatoid arthritis. In addition, anti-human TNF was demonstrated to prevent the development of arthritis in a transgenic human TNF-a mouse model. Elliott et al. (Arthritis Rheum. 1993, 36, 1681-1690) used chimeric (mouse-human) antibodies to TNF-a to treat patients with active rheumatoid arthritis. But, TNF-a might not be the only factor responsible for this inflammatory disease.

Therefore, there is a need for alternative treatments of these diseases that would be more effective on the long term in regards to the above mentioned observations and which would be well tolerated especially in respect to repeated administration.

In connection with the invention, we examined the distribution of mast cell subsets and their density in synovium from normal subjects and from patients with rheumatoid arthritis (RA). In normal synovium, the majority of mast cells belongs to the MCTC subset, outnumbering MCT cells by 5:1. The mean density of mast cells was significantly increased in RA synovia compared with normal synovia. In RA, both subsets expanded and were associated with infiltrating inflammatory cells or with regions of highly cellular fibrous tissue (mainly MCTC). In addition, a correlation was observed between clinical parameters of activity or progression of rheumatoid disease and the density of MCTC cells, especially the density in the superficial layer of synovium.

As a result, MCTC mast cells expand in RA, associate with regions of "active" fibrosis, and correlate with parameters of disease activity or progression of RA. These findings implicate the MCTC subset of mast cells in the pathologic mechanisms that mediate
5 tissue damage in RA.

Mast cells (MC) are tissue elements derived from a particular subset of hematopoietic stem cells that express CD34, c-kit and CD13 antigens (Kirshenbaum et al, Blood. 94: 2333-2342, 1999 and Ishizaka et al, Curr Opin Immunol. 5: 937-43, 1993). Immature
10 MC progenitors circulate in the bloodstream and differentiate in tissues. These differentiation and proliferation processes are under the influence of cytokines, one of utmost importance being Stem Cell Factor (SCF), also termed Kit ligand (KL), Steel factor (SL) or Mast Cell Growth Factor (MCGF). SCF receptor is encoded by the protooncogene c-kit, that belongs to type III receptor tyrosine kinase subfamily (Boissan and Arock, J Leukoc Biol. 67: 135-48, 2000). This receptor is also expressed on others hematopoietic or non hematopoietic cells. Ligation of c-kit receptor by SCF induces its dimerization followed by its transphosphorylation, leading to the recruitment and activation of various intracytoplasmic substrates. These activated substrates induce multiple intracellular signaling pathways responsible for cell proliferation and activation
15 (Boissan and Arock, 2000). Mast cells are characterized by their heterogeneity, not only regarding tissue location and structure but also at the functional and histochemical levels (Aldenborg and Enerback., Histochem. J. 26: 587-96, 1994 ; Bradding et al. J Immunol. 155: 297-307, 1995 ; Irani et al, J Immunol. 147: 247-53, 1991 ; Miller et al, Curr Opin Immunol. 1: 637-42, 1989 and Welle et al, J Leukoc Biol. 61: 233-45, 1997).

25

In connection with the invention, it is proposed that mast cells play a crucial role in the pathogenesis of inflammatory diseases associated with mast cells, such as rheumatoid

arthritis, conjunctivitis, rheumatoid spondylitis, osteoarthritis, gouty arthritis and other arthritic conditions, in that they produce a large variety of mediators categorized here into three groups:

- preformed granule-associated mediators (histamine, proteoglycans, and neutral proteases),
5
- lipid-derived mediators (prostaglandins, thromboxanes and leucotrienes),
- and various cytokines (IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, TNF-a, GM-CSF, MIP-1a, MIP-1b and IFN- γ).

- 10 Then, liberation by activated mast cells of mediators (TNF- a, leucotrienes, prostaglandines etc...) can induce these inflammatory diseases. In addition, mast cells activatie T cells and macrophages, which further contributes to the inflammation and destruction process observed for example in rheumatoid arthritis.
- 15 Therefore, the invention provides a new therapeutic strategy aimed at blocking the activation of mast cells involved in RA.

In US 6,211,228, tryptase inhibitors are proposed for the prevention and treatment of mast-cell mediated inflammatory disorders and in US 5,861,264 anti-tryptase detection
20 is used as a diagnostic for inflammatory diseases. However, decreasing the activity of free tryptase released by activated mast cells is not sufficient to block chain reactions caused by the others mast cells released factors mentioned above.

25 In contrast, the present invention proposes to use c-kit specific kinase inhibitors to inhibit mast cell proliferation, survival and activation. A new route for treating

inflammatory diseases is provided, which consists of destroying mast cells playing a role in the pathogenesis of these disorders. It has been found that tyrosine kinase inhibitors and more particularly c-kit inhibitors are especially suited to reach this goal.

5 **Description**

The present invention relates to a method for treating inflammatory diseases comprising administering a tyrosine kinase inhibitor to a mammal in need of such treatment.

- 10 Tyrosine kinase inhibitors are selected for example from bis monocyclic, bicyclic or heterocyclic aryl compounds (WO 92/20642), vinylene-azaindole derivatives (WO 94/14808) and 1-cyclopropyl-4-pyridyl-quinolones (US 5,330,992), Styryl compounds (US 5,217,999), styryl-substituted pyridyl compounds (US 5,302,606), seleoindoles and selenides (WO 94/03427), tricyclic polyhydroxylic compounds (WO 92/21660) and
15 benzylphosphonic acid compounds (WO 91/15495), pyrimidine derivatives (US 5,521,184 and WO 99/03854), indolinone derivatives and pyrrol-substituted indolinones (US 5,792,783, EP 934 931, US 5,834,504, US 5,883,116, US 5,883,113, US 5,
886,020, WO 96/40116 and WO 00/38519), as well as bis monocyclic, bicyclic aryl and heteroaryl compounds (EP 584 222, US 5,656,643 and WO 92/20642), quinazoline
20 derivatives (EP 602 851, EP 520 722, US 3,772,295 and US 4,343,940) and aryl and heteroaryl quinazoline (US 5,721,237, US 5,714,493, US 5,710,158 and WO 95/15758).

Preferably, said tyrosine kinase inhibitors are unable to promote death of IL-3 dependent cells cultured in presence of IL-3.

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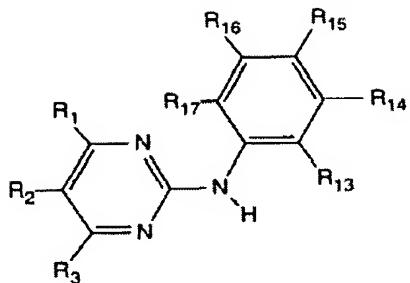
In another embodiment, the invention is directed to a method for treating inflammatory diseases comprising administering a c-kit inhibitor to a mammal in need of such treatment.

Preferably, said c-kit inhibitor is a non-toxic, selective and potent c-kit inhibitor. Such inhibitors can be selected from the group consisting of indolinones, pyrimidine derivatives, pyrrolopyrimidine derivatives, quinazoline derivatives, quinoxaline derivatives, pyrazoles derivatives, bis monocyclic, bicyclic or heterocyclic aryl compounds, vinylene-azaindole derivatives and pyridyl-quinolones derivatives, styryl compounds, styryl-substituted pyridyl compounds, seleoindoles, selenides, tricyclic polyhydroxylic compounds and benzylphosphonic acid compounds.

Among preferred compounds, it is of interest to focus on pyrimidine derivatives such as
10 N-phenyl-2-pyrimidine-amine derivatives (US 5,521,184 and WO 99/03854), indolinone derivatives and pyrrol-substituted indolinones (US 5,792,783, EP 934 931, US 5,834,504), US 5,883,116, US 5,883,113, US 5, 886,020, WO 96/40116 and WO 00/38519), as well as bis monocyclic, bicyclic aryl and heteroaryl compounds (EP 584 222, US 5,656,643 and WO 92/20642), quinazoline derivatives (EP 602 851, EP 520 15 722, US 3,772,295 and US 4,343,940), 4-amino-substituted quinazolines (US 3,470,182), 4-thienyl-2-(1H)-quinazolones, 6,7-dialkoxyquinazolines (US 3,800,039), aryl and heteroaryl quinazoline (US 5,721,237, US 5,714,493, US 5,710,158 and WO 95/15758), 4-anilinoquinazoline compounds (US 4,464,375), and 4-thienyl-2-(1H)-quinazolones (US 3,551,427).

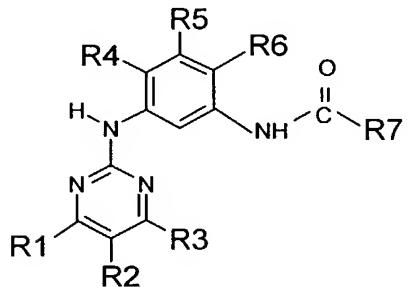
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So, preferably, the invention relates to a method for treating inflammatory diseases comprising administering a non toxic, potent and selective c-kit inhibitor. Such inhibitor can be selected from pyrimidine derivatives, more particularly N-phenyl-2-pyrimidine-amine derivatives of formula I :



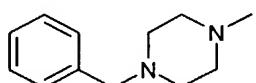
wherein the R1, R2, R3, R13 to R17 groups have the meanings depicted in EP 564 409 B1, incorporated herein in the description.

- 5 Preferably, the N-phenyl-2-pyrimidine-amine derivative is selected from the compounds corresponding to formula II :



- 10 Wherein R1, R2 and R3 are independently chosen from H, F, Cl, Br, I, a C1-C5 alkyl or a cyclic or heterocyclic group, especially a pyridyl group;
R4, R5 and R6 are independently chosen from H, F, Cl, Br, I, a C1-C5 alkyl, especially a methyl group;
and R7 is a phenyl group bearing at least one substituent, which in turn possesses at least
15 one basic site, such as an amino function.

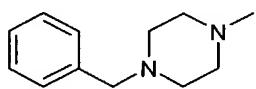
Preferably, R7 is the following group :



Among these compounds, the preferred are defined as follows :

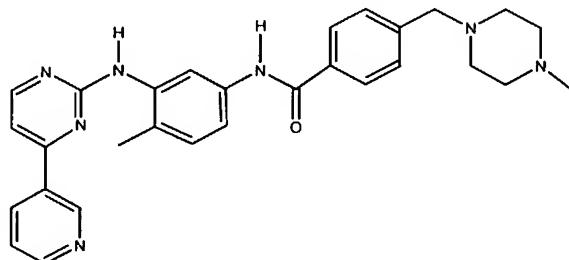
- R1 is a heterocyclic group, especially a pyridyl group,
- R2 and R3 are H,
- 5 R4 is a C1-C3 alkyl, especially a methyl group,
- R5 and R6 are H,
- and R7 is a phenyl group bearing at least one substituent, which in turn possesses at least one basic site, such as an amino function, for example the group :

10



Therefore, in a preferred embodiment, the invention relates to a method for treating inflammatory diseases comprising the administration of an effective amount of the compound known in the art as CGP57148B :

- 15 4-(4-méthylpipérazine-1-ylméthyl)-N-[4-méthyl-3-(4-pyridine-3-yl)pyrimidine-2-ylamino]phényl]-benzamide corresponding to the following formula :



The preparation of this compound is described in example 21 of EP 564 409 and the β -form, which is particularly useful is described in WO 99/03854.

Alternatively, the c-kit inhibitor can be selected from :

- indolinone derivatives, more particularly pyrrol-substituted indolinones,
- monocyclic, bicyclic aryl and heteroaryl compounds, quinazoline derivatives,
- and quinaxolines, such as 2-phényl-quinaxoline derivatives, for example 2-phenyl-
5 6,7-dimethoxy quinaxoline.

In a preferred aspect, the invention contemplated the method mentioned above, wherein said c-kit inhibitor is unable to promote death of IL-3 dependent cells cultured in presence of IL-3.

10

The expression "inflammatory diseases" refers herein to inflammatory diseases associated with mast cells, such as rheumatoid arthritis, conjunctivitis, rheumatoid spondylitis, osteoarthritis, gouty arthritis and other arthritic conditions, which are embraced by the invention.

15

In a further embodiment, c-kit inhibitors as mentioned above are inhibitors of activated c-kit. In frame with the invention, the expression "activated c-kit" means a constitutively activated-mutant c-kit including at least one mutation selected from point mutations, deletions, insertions, but also modifications and alterations of the natural c-kit sequence
20 (SEQ ID N°1). Such mutations, deletions, insertions, modifications and alterations can occur in the transphosphorylase domain, in the juxtamembrane domain as well as in any domain directly or indirectly responsible for c-kit activity. The expression "activated c-kit" also means herein SCF-activated c-kit. Preferred and optimal SCF concentrations for activating c-kit are comprised between 5.10^{-7} M and 5.10^{-6} M, preferably around
25 2.10^{-6} M. In a preferred embodiment, the activated-mutant c-kit in step a) has at least one mutation proximal to Y823, more particularly between amino acids 800 to 850 of SEQ ID No1 involved in c-kit autophosphorylation, notably the D816V, D816Y, D816F and D820G mutants. In another preferred embodiment, the activated-mutant c-kit in step a)

has a deletion in the juxtamembrane domain of c-kit. Such a deletion is for example between codon 573 and 579 called c-kit d(573-579). The point mutation V559G proximal to the juxtamembrane domain c-kit is also of interest.

5 In this regard, the invention contemplates a method for treating inflammatory diseases comprising administering to a mammal in need of such treatment a compound that is a selective, potent and non toxic inhibitor of activated c-kit obtainable by a screening method which comprises :

a) bringing into contact (i) activated c-kit and (ii) at least one compound to be tested;
10 under conditions allowing the components (i) and (ii) to form a complex,
b) selecting compounds that inhibit activated c-kit,
c) testing and selecting a subset of compounds identified in step b), which are unable to promote death of IL-3 dependent cells cultured in presence of IL-3.

15 This screening method can further comprise the step consisting of testing and selecting a subset of compounds identified in step b) that are inhibitors of mutant activated c-kit (for example in the transphosphorylase domain), which are also capable of inhibiting SCF-activated c-kit wild.

Alternatively, in step a) activated c-kit is SCF-activated c-kit wild.

20

A best mode for practicing this method consists of testing putative inhibitors at a concentration above 10 µM in step a). Relevant concentrations are for example 10, 15, 20, 25, 30, 35 or 40 µM.

25 In step c), IL-3 is preferably present in the culture media of IL-3 dependent cells at a concentration comprised between 0.5 and 10 ng/ml, preferably between 1 to 5 ng/ml.

Examples of IL-3 dependent cells include but are not limited to :

- cell lines naturally expressing and depending on c-kit for growth and survival. Among such cells, human mast cell lines can be established using the following procedures : normal human mast cells can be infected by retroviral vectors containing sequences coding for a mutant c-kit comprising the c-kit signal peptide and a TAG sequence 5 allowing to differentiate mutant c-kits from c-kit wild expressed in hematopoietic cells by means of antibodies.

This technique is advantageous because it does not induce cellular mortality and the genetic transfer is stable and gives satisfactory yields (around 20 %). Pure normal human mast cells can be routinely obtained by culturing precursor cells originating from blood 10 obtained from human umbilical vein. In this regard, heparinated blood from umbilical vein is centrifuged on a Ficoll gradient so as to isolate mononucleated cells from other blood components. CD34+ precursor cells are then purified from the isolated cells mentioned above using the immunomagnetic selection system MACS (Miltenyi biotech). CD34+ cells are then cultured at 37°C in 5 % CO₂ atmosphere at a concentration of 10⁵ 15 cells per ml in the medium MCCM (α -MEM supplemented with L-glutamine, penicillin, streptomycin, 5 10⁻⁵ M β -mercaptoethanol, 20 % veal foetal serum, 1 % bovine albumin serum and 100 ng/ml recombinant human SCF. The medium is changed every 5 to 7 days. The percentage of mast cells present in the culture is assessed each week, using May-Grünwald Giemsa or Toluidine blue coloration. Anti-tryptase antibodies can also be 20 used to detect mast cells in culture. After 10 weeks of culture, a pure cellular population of mast cells (> 98 %) is obtained.

It is possible using standard procedures to prepare vectors expressing c-kit for transfecting the cell lines established as mentioned above. The cDNA of human c-kit has been described in Yarden et al., (1987) EMBO J.6 (11), 3341-3351. The coding part of

c-kit (3000 bp) can be amplified by PCR and cloned, using the following oligonucleotides :

- 5'AAGAAGAGATGGTACCTCGAGGGGTGACCC3' (SEQ ID No2) sens
 - 5'CTGCTTCGCAGGCCGCGTTAACTCTCTCAACCA3' (SEQ ID No3)
- 5 antisens
- The PCR products, digested with Not1 and Xho1, has been inserted using T4 ligase in the pFlag-CMV vector (SIGMA), which vector is digested with Not1 and Xho1 and dephosphorylated using CIP (Biolabs). The pFlag-CMV-c-kit is used to transform bacterial clone XL1-blue. The transformation of clones is verified using the following 10 primers :

- 5'AGCTCGTTAGTGAACCGTC3' (SEQ ID No4) sens,
- 5'GTCAGACAAAATGATGCAAC3' (SEQ ID No5) antisens.

Directed mutagenesis is performed using relevant cassettes is performed with routine and common procedure known in the art..

- 15 The vector Migr-1 (ABC) can be used as a basis for constructing retroviral vectors used for transfecting mature mast cells. This vector is advantageous because it contains the sequence coding for GFP at the 3' and of an IRES. These features allow to select cells infected by the retrovirus using direct analysis with a fluorocytometer. As mentioned above, the N-terminal sequence of c-kit c-DNA can be modified so as to introduce a Flag 20 sequence that will be useful to discriminating heterogeneous from endogenous c-kit.

Other IL-3 dependent cell lines that can be used include but are not limited to:

- BaF3 mouse cells expressing wild-type or mutated form of c-kit (in the juxtamembrane and in the catalytic sites) are described in Kitayama et al, (1996), Blood 25 88, 995-1004 and Tsujimura et al, (1999), Blood 93, 1319-1329.

- IC-2 mouse cells expressing either c-kit^{WT} or c-kit^{D814Y} are presented in Piao et al, (1996), Proc. Natl. Acad. Sci. USA 93, 14665-14669.

IL-3 independent cell lines are :

- 5 - HMC-1, a factor-independent cell line derived from a patient with mast cell leukemia, expresses a juxtamembrane mutant c-kit polypeptide that has constitutive kinase activity (Furitsu T et al, J Clin Invest. 1993;92:1736-1744 ; Butterfield et al, Establishment of an immature mast cell line from a patient with mast cell leukemia. Leuk Res. 1988;12:345-355 and Nagata et al, Proc Natl Acad Sci U S A. 1995;92:10560-10564).
- 10 - P815 cell line (mastocytoma naturally expressing c-kit mutation at the 814 position) has been described in Tsujimura et al, (1994), Blood 83, 2619-2626.

The extent to which component (ii) inhibits activated c-kit can be measured *in vitro* or *in vivo*. In case it is measured *in vivo*, cell lines expressing an activated-mutant c-kit, which 15 has at least one mutation proximal to Y823, more particularly between amino acids 800 to 850 of SEQ ID No1 involved in c-kit autophosphorylation, notably the D816V, D816Y, D816F and D820G mutants, are preferred.

Example of cell lines expressing an activated-mutant c-kit are as mentioned.

- 20 In another preferred embodiment, the method further comprises the step consisting of testing and selecting compounds capable of inhibiting c-kit wild at concentration below 1 μM. This can be measured *in vitro* or *in vivo*.

In vivo testing may comprise measuring the ability of the tyrosine kinase inhibitors to 25 alleviate arthritis symptoms in transgenic mouse model of arthritis that spontaneously develops a disease with many of the characteristics of rheumatoid arthritis in humans

(Kouskoff et al., 1996 and US 5,675,060). Alternatively, transgenic human TNF-a mouse model can be used.

Therefore, compounds are identified and selected according to the method described
5 above are potent, selective and non-toxic c-kit wild inhibitors.

Alternatively, the screening method as defined above can be practiced *in vitro*. In this regard, the inhibition of mutant-activated c-kit and/or c-kit wild can be measured using standard biochemical techniques such as immunoprecipitation and western blot.
10 Preferably, the amount of c-kit phosphorylation is measured.

In a still further embodiment, the invention contemplates a method for treating inflammatory diseases as depicted above wherein the screening comprises :

- a) performing a proliferation assay with cells expressing a mutant c-kit (for example in
15 the transphosphorylase domain), which mutant is a permanent activated c-kit, with a plurality of test compounds to identify a subset of candidate compounds targeting activated c-kit, each having an IC₅₀ < 10 μM, by measuring the extent of cell death,
- b) performing a proliferation assay with cells expressing c-kit wild said subset of candidate compounds identified in step (a), said cells being IL-3 dependent cells cultured
20 in presence of IL-3, to identify a subset of candidate compounds targeting specifically c-kit,
- c) performing a proliferation assay with cells expressing c-kit, with the subset of compounds identified in step b) and selecting a subset of candidate compounds targeting c-kit wild, each having an IC₅₀ < 10 μM, preferably an IC₅₀ < 1 μM, by measuring the
25 extent of cell death.

Here, the extent of cell death can be measured by ^3H thymidine incorporation, the trypan blue exclusion method or flow cytometry with propidium iodide. These are common techniques routinely practiced in the art.

- 5 The method according to the invention includes preventing, delaying the onset and/or treating inflammatory diseases in human.

Therefore, the invention embraces the use of the compounds defined above to manufacture a medicament for treating inflammatory diseases such as rheumatoid 10 arthritis, conjunctivitis, rheumatoid spondylitis, osteoarthritis, gouty arthritis, polyarthritis, and other arthritic conditions as well as pain associated with these inflammatory diseases.

15 The pharmaceutical compositions utilized in this invention may be administered by any number of routes including, but not limited to oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, or rectal means.

In addition to the active ingredients, these pharmaceutical compositions may contain 20 suitable pharmaceutically-acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Further details on techniques for formulation and administration may be found in the latest edition of Remington's Pharmaceutical Sciences (Maack Publishing Co., Easton, Pa.).

25

Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated

as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

Pharmaceutical compositions suitable for use in the invention include compositions wherein c-kit inhibitors are contained in an effective amount to achieve the intended purpose. The determination of an effective dose is well within the capability of those skilled in the art. A therapeutically effective dose refers to that amount of active ingredient, which ameliorates the symptoms or condition. Therapeutic efficacy and toxicity may be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., ED50 (the dose therapeutically effective in 50% of the population) and LD50 (the dose lethal to 50% of the population). The dose ratio of toxic to therapeutic effects is the therapeutic index, and it can be expressed as the ratio, LD50/ED50. Pharmaceutical compositions which exhibit large therapeutic indices are preferred. As mentioned above, a tyrosine kinase inhibitor and more particularly a c-kit inhibitor according to the invention is unable to promote death of IL-3 dependent cells cultured in presence of IL-3.

The invention also concerns a product comprising a tyrosine kinase inhibitor as defined above and at least one anti-inflammatory compound selected from the group consisting of acetylsalicylic acid (aspirin), ibuprofen, naproxen, and cox2 inhibitors.

Utility of the invention will further ensue from the detailed description below.

Exemple 1: Use of 4-(4-méthylpipérazine-1-ylméthyl)-N-[4-méthyl-3-(4-pyridine-3-yl)pyrimidine-2 ylamino]phényl]-benzamide for treating arthritis.

Protocol Treatment

The mice were pretreated with the compound at different concentrations for two days (day-2, day -1) before induction of arthritis. Arthritis was induced by ip injection of 150 µl serum at days 0 and 2. The treatment with the compound at different concentration was continued during the course of the disease. The control mice were injected with acid PBS before the induction of arthritis and during the course of the disease. Ankle thickness and arthritis score was evaluated for 12 days. Arthritis Score : Sum of scores of each limb (0 no disease; 0.5 mild swelling of paw or of just a few digits; 1 clear joint inflammation; max score=4). The results are presented in **Figures 1 and 2**. Table 1 shows the number of mice used in this study.

10 Table 1

Compound concentration	BalbC/cj	C57Bl/6
0.5 mg	2	
1 mg		2
1.5 mg		2
2mg		
3 mg	2	

Controls of the treated mice	BalbC/cj	C57Bl/6
0.5 mg	3	
1 mg		2
1.5 mg		2
2 mg		
*3 mg	2	

15 *The dose of 3mg was lethal.
The compound was dissolved in acid PBS solution, pH 3. In order to rule out any toxic effect of this low pH studies on the natural activation of B and T cells was monitored. 3 mice were injected with acid PBS for 4 days and compared to 3 control mice that were injected with PBS.

20

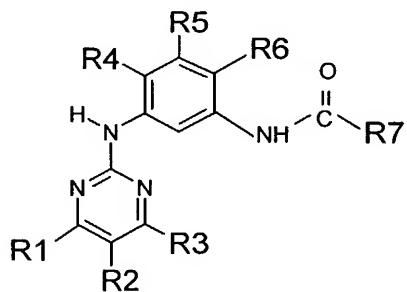
Histology

At the end of the experiment the hind limbs were collected. The skin of the limb was removed and the limbs were subsequently fixed in 2% paraformaldehyde.

CLAIMS

1. A method for treating inflammatory diseases comprising administering a tyrosine kinase inhibitor to a mammal in need of such treatment.
5
2. A method according to claim 1, wherein said tyrosine kinase inhibitor is unable to promote death of IL-3 dependent cells cultured in presence of IL-3.
3. A method for treating inflammatory diseases comprising administering a c-kit inhibitor to a mammal in need of such treatment.
10
4. A method according to claim 3, wherein said c-kit inhibitor is a non-toxic, selective and potent c-kit inhibitor.
- 15 5. A method according to claim 4, wherein said inhibitor is selected from the group consisting of indolinones, pyrimidine derivatives, pyrrolopyrimidine derivatives, quinazoline derivatives, quinoxaline derivatives, pyrazoles derivatives, bis monocyclic, bicyclic or heterocyclic aryl compounds, vinylene-azaindole derivatives and pyridyl-quinolones derivatives, styryl compounds, styryl-substituted pyridyl compounds,
20 seleoindoles, selenides, tricyclic polyhydroxylic compounds and benzylphosphonic acid compounds.
6. A method according to claim 4, wherein said inhibitor is selected from the group consisting of :
25
 - pyrimidine derivatives, more particularly N-phenyl-2-pyrimidine-amine derivatives,
 - indolinone derivatives, more particularly pyrrol-substituted indolinones,
 - monocyclic, bicyclic aryl and heteroaryl compounds,
 - and quinazoline derivatives.

7. A method according to one of claims 1 to 6, wherein said inhibitor is selected from the group consisting of N-phenyl-2-pyrimidine-amine derivatives having the formula II :

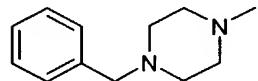


5

Wherein R1, R2 and R3 are independently chosen from H, F, Cl, Br, I, a C1-C5 alkyl or a cyclic or heterocyclic group, especially a pyridyl group;

R4, R5 and R6 are independently chosen from H, F, Cl, Br, I, a C1-C5 alkyl, especially a methyl group;

10 and R7 is a phenyl group bearing at least one substituent, which in turn possesses at least one basic site, such as an amino function, preferably the following group :



15 8. A method according to claim 7, wherein said inhibitor is the 4-(4-méthylpipérazine-1-ylméthyl)-N-[4-méthyl-3-(4-pyridine-3-yl)pyrimidine-2 ylamino]phényl]-benzamide.

9. A method according to one of claims 3 to 8, wherein said c-kit inhibitor is unable to promote death of IL-3 dependent cells cultured in presence of IL-3.

20 10. A method according to one of claims 3 to 9, wherein said c-kit inhibitor is an inhibitor of activated c-kit.

11. A method according to claim 10, wherein said activated c-kit inhibitor is capable of inhibiting SCF-activated c-kit.

12. A method according to claim 10, wherein said inhibitor is capable of inhibiting
5 constitutively activated-mutant c-kit.

13. A method for treating inflammatory diseases comprising administering to a mammal in need of such treatment a compound that is a selective, potent and non toxic inhibitor of activated c-kit obtainable by a screening method which comprises :

- 10 a) bringing into contact (i) activated c-kit and (ii) at least one compound to be tested; under conditions allowing the components (i) and (ii) to form a complex,
 b) selecting compounds that inhibit activated c-kit,
 c) testing and selecting a subset of compounds identified in step b), which are unable to promote death of IL-3 dependent cells cultured in presence of IL-3.

15

14. A method according to claim 13, wherein the screening method further comprises the step consisting of testing and selecting a subset of compounds identified in step b) that are inhibitors of mutant activated c-kit, which are also capable of inhibiting SCF-activated c-kit wild.

20

15. A method according to claim 13, wherein activated c-kit is SCF-activated c-kit wild in step a).

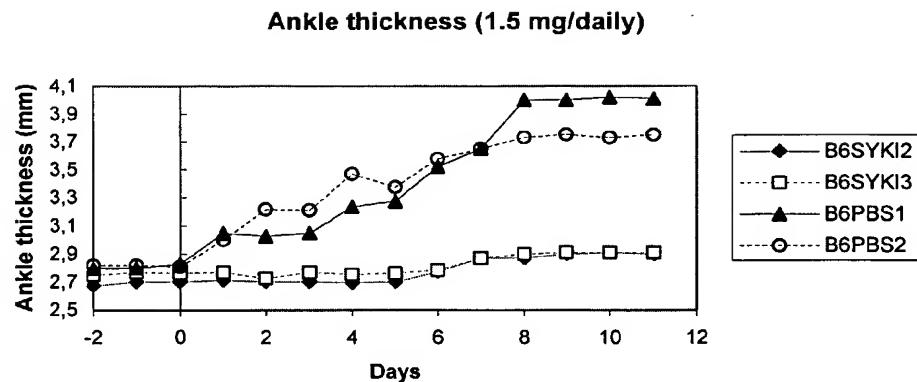
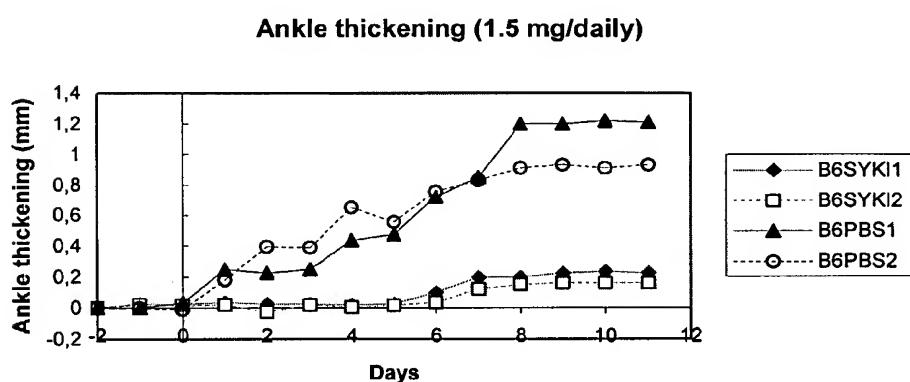
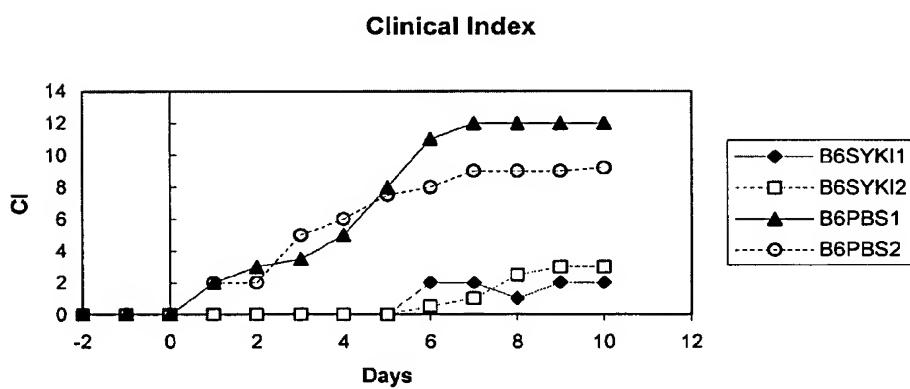
25 16. A method according to one of claims 13 to 15, wherein putative inhibitors are tested at a concentration above 10 µM in step a).

17. A method according to one of claims 13 to 16, wherein IL-3 is preferably present in the culture media of IL-3 dependent cells at a concentration comprised between 0.5 and 10 ng/ml, preferably between 1 to 5 ng/ml.
- 5 18. A method according to claim 17, wherein IL-3 dependent cells are selected from the group consisting of mast cells, transfected mast cells, BaF3, and IC-2.
19. A method according to one of claims 13 to 17, wherein the extent to which component (ii) inhibits activated c-kit is measured *in vitro* or *in vivo*.
- 10 20. A method according to one of claims 13 to 17, further comprising the step consisting of testing and selecting compounds capable of inhibiting c-kit wild at concentration below 1 μ M.
- 15 21. A method according to claim 20, wherein the testing is performed *in vitro* or *in vivo*.
22. A method according to one of claims 13 to 21, wherein the inhibition of mutant-activated c-kit and/or c-kit wild is measured using standard biochemical techniques such as immunoprecipitation and western blot.
- 20 23. A method according to one of claims 13 to 22, wherein the amount of c-kit phosphorylation is measured.
24. A method according to one of claims 13 to 23, wherein identified and selected compounds are potent, selective and non-toxic c-kit wild inhibitors.
- 25 25. A method for treating inflammatory diseases comprising administering to a mammal in need of such treatment a c-kit inhibitor obtainable by a screening method comprising :

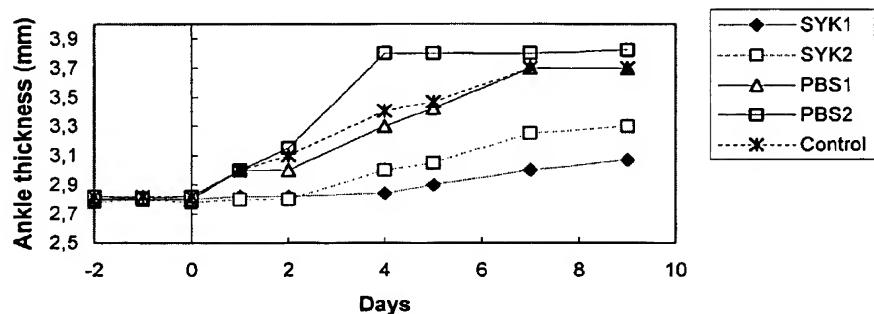
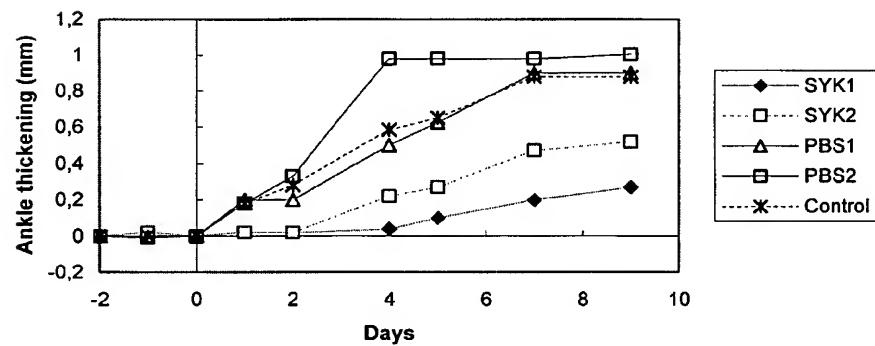
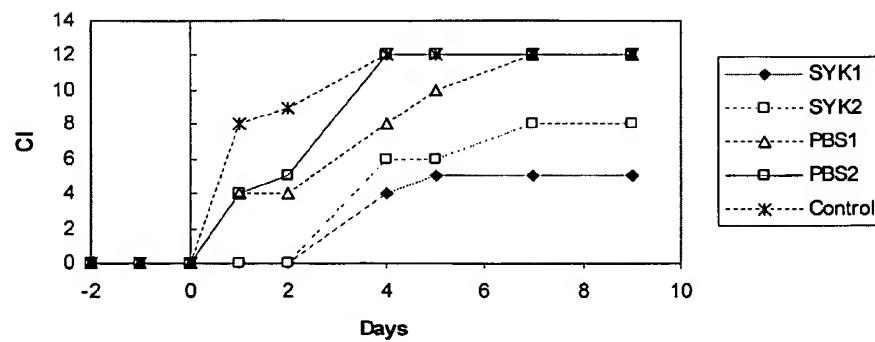
- a) performing a proliferation assay with cells expressing a mutant c-kit (for example in the transphosphorylase domain), which mutant is a permanent activated c-kit, with a plurality of test compounds to identify a subset of candidate compounds targeting activated c-kit, each having an IC₅₀ < 10 μM, by measuring the extent of cell death,
- 5 b) performing a proliferation assay with cells expressing c-kit wild said subset of candidate compounds identified in step (a), said cells being IL-3 dependent cells cultured in presence of IL-3, to identify a subset of candidate compounds targeting specifically c-kit,
- c) performing a proliferation assay with cells expressing c-kit, with the subset of
10 compounds identified in step b) and selecting a subset of candidate compounds targeting c-kit wild, each having an IC₅₀ < 10 μM, preferably an IC₅₀ < 1 μM, by measuring the extent of cell death.
26. A method according to claim 25, wherein the extent of cell death is measured by ³H thymidine incorporation, the trypan blue exclusion method or flow cytometry with propidium iodide.
15
27. A method according to one of claims 1 to 26 for preventing, delaying the onset and/or treating inflammatory diseases in human.
20
28. A method according to one of claims 1 to 26 for preventing, delaying the onset and/or treating an inflammatory disease selected from the group consisting of rheumatoid arthritis, conjunctivitis, rheumatoid spondylitis, osteoarthritis, gouty arthritis, polyarthritis, and other arthritic conditions as well as pain associated with these
25 inflammatory diseases.
29. Use of a c-kit inhibitor to manufacture a medicament for treating inflammatory diseases.

30. A composition suitable for oral administration comprising a tyrosine kinase inhibitor, more particularly a c-kit inhibitor for the treatment of inflammatory disorders such as rheumatoid arthritis, conjunctivitis, rheumatoid spondylitis, osteoarthritis, gouty arthritis, 5 polyarthritis, and other arthritic conditions as well as pain associated with these inflammatory diseases.
31. A composition suitable for topical, intranasal, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, 10 intraperitoneal, enteral, sublingual, or rectal administration comprising a tyrosine kinase inhibitor, more particularly a c-kit inhibitor for the treatment of inflammatory disorders such as rheumatoid arthritis, conjunctivitis, rheumatoid spondylitis, osteoarthritis, gouty arthritis, polyarthritis, and other arthritic conditions as well as pain associated with these inflammatory diseases.

1 / 2

**FIGURE 1A****FIGURE 1B****FIGURE 1C**

2 / 2

Ankle thickness (0.5mg/daily)**FIGURE 2A****Ankle thickening (0.5mg/daily)****FIGURE 2B****Clinical Index****FIGURE 2C**

SEQUENCE LISTING

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<120> Use of tyrosine kinase inhibitors for treating
inflammatory diseases

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<150> US 60/301,410
<151> 2001-06-29

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Declarations under Rule 4.17:

- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZM, ZW, ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)
- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii)) for all designations of inventorship (Rule 4.17(iv)) for US only

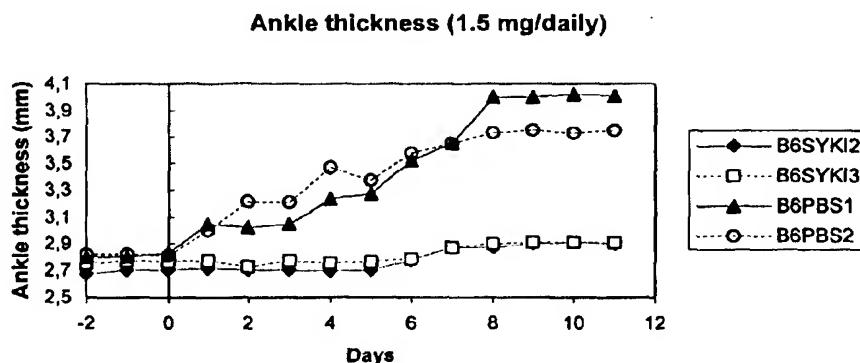
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[Continued on next page]

(54) Title: USE OF TYROSINE KINASE INHIBITORS FOR TREATING INFLAMMATORY DISEASES



WO 03/002108 A3

(57) Abstract: The present invention relates to a method for treating inflammatory diseases such as rheumatoid arthritis (RA), comprising administering a tyrosine kinase inhibitor to a human in need of such treatment, more particularly a non-toxic, selective and potent c-kit inhibitor. Preferably, said inhibitor is unable to promote death of IL-3 dependent cells cultured in presence of IL-3.



For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

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Inte
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PL 02/03301

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K31/00 A61K31/404 A61K31/506 A61K31/505 A61K31/519
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 A61K31/095 A61K31/66 A61P29/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

CHEM ABS Data, SCISEARCH, EMBASE, MEDLINE, BIOSIS, EPO-Internal, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 01 45689 A (SUGEN INC ;LIPSON KEN (US); MCMAHON GERALD (US)) 28 June 2001 (2001-06-28) page 4, line 10 - line 19 page 23, line 14 - line 17 ---	1-6, 9-12, 27-31
X	US 5 792 783 A (TANG PENG CHO ET AL) 11 August 1998 (1998-08-11) claims 15-17,20-24,27 ---	1-6, 9-12, 27-31
X	WO 00 56709 A (MILLER TODD ANTHONY ;LIANG CONGXIN (US); SUGEN INC (US); TANG PENG) 28 September 2000 (2000-09-28) claims 1,7,18,21,22,26-28,34,35,37-39 --- -/-	1-6, 9-12, 27-31

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

° Special categories of cited documents :

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- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"&" document member of the same patent family

Date of the actual completion of the international search

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Authorized officer

van der Kooij, M

INTERNATIONAL SEARCH REPORT

International Application No

PCT/IB 02/03301

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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